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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	249/127
First named Inventor or Application Identifier	Dr. Paddy Jim Baggot
Title	TREATMENT OF CHROMOSOMAL ABNORMALITIES IN FETUSES THROUGH A COMPREHENSIVE METABOLIC ANALYSIS OF AMNIOTIC FLUID
Express Mail Label No.	EL 496515405US

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contacts

ADDRESS TO: **Assistant Commissioner for Patents**
Box Patent Application
Washington, D.C. 20231

1. ☐ Fee Transmittal Form
(Submit an original, and a duplicate for fee processing)
2. + Specification [*Total Pages* 24]
(preferred arrangement set forth below)
-Descriptive title of the Invention
-Cross References to Related Applications
-Statement Regarding Fed sponsored R&D
-Reference to Microfiche Appendix
-Background of the Invention
-Brief Summary of the Invention
-Brief Description of the Drawings (if filed)
-Detailed Description
-Claim(s)
-Abstract of the Disclosure
3. ☐ Drawing(s) (35 USC § 113) [Total Sheets]
4. ☐ Oath or Declaration [Total Sheets]
a. ☐ Newly executed (original or copy)
b. ☐ Copy from a prior application (37 CFR 1.63(d))
(for continuation/divisional with Box 17 completed)
(Note Box 5 below)
i. ☐ DELETION OF INVENTORS
Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.53(d)(2) and 1.53(b).

NOTE FOR ITEMS 1 & 13 IN ORDER TO BE ENTITLED TO PAY SMALL ENTITY FEES, A SMALL ENTITY STATEMENT IS REQUIRED (37 C F R § 1 27), EXCEPT IF ONE FILED IN A PRIOR APPLICATION IS RELIED UPON (37 C F R. §1.28)

5. ☐ Microfiche Computer Program (Appendix)

6. Nucleotide and/or Amino Acid Sequence Submission
(If applicable, all necessary)
a. ☐ Computer readable Copy
b. ☐ Paper Copy (Identical to computer copy)
c. ☐ Statement verifying identity of above copies

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ACCOMPANYING APPLICATION PARTS

7. ☐ Assignment Papers (cover sheet & document(s))
8. ☐ 37 CFR 3.73(b) Statement + Power of Attorney
(when there is an assignment)
9. ☐ English Translation Document (if applicable)
10. ☐ Information Disclosure ☐ Copies of IDS
Statement (IDS) PTO-1449 Citations
11. ☐ Preliminary Amendment
12. + Return Receipt Postcard (MPEP 503)
(Should be specifically itemized)
13. ☐ Small Entity Statement filed in prior application
Statement(s) Status still proper and desired
14. ☐ Certified Copy of Priority Document(s)
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16. **If a CONTINUING APPLICATION**, check appropriate box and supply the requisite information below and in a preliminary amendment.
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For CONTINUATION or DIVISIONAL APPS only: The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 4b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

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OC-44431.1

TITLE

TREATMENT OF CHROMOSOMAL ABNORMALITIES IN FETUSES THROUGH A COMPREHENSIVE METABOLIC ANALYSIS OF AMNIOTIC FLUID

FIELD OF THE INVENTION

The present invention relates to the diagnosis of biochemical abnormalities found in a fetus. Specifically, the present invention is a comprehensive metabolic profile of an amniotic fluid specimen to diagnose and prescribe treatment for chromosomal disorders, preferably Down's Syndrome, that may be present in a fetus.

BACKGROUND

Chromosomal abnormalities are forms of birth defects that occur in 2 to 3 out of every 1000 births. Chromosome abnormalities may involve duplications or defects in a whole chromosome or a portion of one or more chromosomes. The most common chromosome abnormality is Down Syndrome. In Down Syndrome, each cell contains three copies of chromosome number 21, a condition referred to as trisomy 21. A result of trisomy 21 is a 50% increase in gene dosage for each gene on chromosome 21. At least some of the abnormalities seen in Down Syndrome can be attributed to excessive gene dosage. Down Syndrome is a complicated disorder, affecting many aspects of physiology. Since affected individuals all have mental retardation, it is a significant disability.

Less common chromosomal disorders involve other chromosomes. Some chromosomal disorders result from a deletion or a duplication of a portion of a chromosome. Other

chromosomal disorders result from an absence of an entire chromosome. For example, an absence of the X chromosome results in Turner Syndrome, referred to as monosomy X.

The relationship between chromosome abnormalities and physical or metabolic disorders in children is not always clear. Down Syndrome is the most common and most thoroughly studied chromosome abnormality. This has spurred some authors to look for treatable or correctable abnormalities in children with Down Syndrome. Le Jeune suggested that abnormalities of purine synthesis, thyroid metabolism, and perturbations of enzymes, specifically copper/zinc superoxide dismutase, phosphofructokinase, and cystathione beta synthase, were related to Down Syndrome. Le Jeune, J., *Pathogenesis of Mental Deficiency in Trisomy 21*, Am. J. Med. Genetics Suppl. 7: 20-30 (1990). Additionally, Patterson and Ekvall suggested that a number of vitamins, minerals, and other metabolic abnormalities contributed to Down Syndrome. Patterson, B. and Ekvall, S.W., *Down Syndrome* in *Pediatric Nutrition in Chronic Diseases and Developmental Disorders: Prevention Assessment and Treatment*, Oxford Univ. Press, New York, 149-156 (1993). These theories have not been confirmed by other authors.

Several treatments have been proposed to address Down Syndrome. Turkel advocated the use of "orthomolecular" therapy. Turkel, H.B., *Medical Amelioration of Down Syndrome Incorporating the Orthomolecular Approach*, Psychiatry 4(2): 102-115 (1975). Harrell, et al. suggested supplementation with megadose vitamins. Harrell, et al., *Can Nutritional Supplements Help Mentally Retarded Children?*, PNAS USA 78(1): 574-578 (1981). As reported by Patterson and Ekvall, attempts to verify the effectiveness of the aforementioned treatments have proven unsuccessful. The difficulty of verifying the effectiveness of proposed treatments is compounded since researchers have not confirmed many of the proposed abnormalities. Also, some abnormalities could interact with other abnormalities. Folate metabolism, for example, is

also involved in purine synthesis. Consequently, there is a need for a method to comprehensively analyze a wide panel of metabolites in order to create a complete biochemical picture of a given chromosomal abnormality. Using this method, corresponding treatment may then be identified and prescribed as a result of the complete biochemical picture.

The treatments of Turkel and Harrell et al. might be the right treatment given at the wrong time. Treatment, to be effective, oftentimes must be administered at the proper time. For example, supplementation of folate before and during neural tube development often prevents spina bifida. However, folate supplementation given after birth would be ineffective in preventing spina bifida.

In particular, neurologic development proceeds sequentially. The proper development of a structure depends on previous development of prior structures. Once a structure has developed incorrectly, the error is fixed and cannot later be revised.

For example, the harmful effects of Down Syndrome on brain development begin before birth. A baby with Down Syndrome already has many characteristic features of the disease at the time of birth. There is often hypoplasis of the frontal lobes and cerebellum resulting in a reduced anteroposterior diameter of the head. This is commonly referred to as brachycephaly. The most important events in brain development are the earliest ones, and later developments are less important. As a result, there is a need for a method by which an abnormality, particularly a chromosomal abnormality, may be detected at any early stage of development, such as at the pre-natal stage. A course of treatment may then be prescribed at a time that is beneficial to the baby.

In abnormal fetal development, as in other pathologies, existing techniques can frequently identify a deficiency or abnormality in the existence or metabolism of a physiologically significant species. For example, various methods for analysis of target analytes

generally, and evaluation of analytes in regards to prenatal testing specifically, have been proposed. Examples of some of these methods are disclosed in the following U.S. Patents: 5,326,708, 5,438,017, 5,439,803, 5,506,150, 5,532,131, and 5,670,380.

However, many vitamins are cofactors for multiple enzymes. Similarly, minerals can be cofactors for enzymes. A given metabolite might be a substrate in some reactions and/or a product in other reactions. A determination that a single metabolite, or a single vitamin or mineral is under or over-expressed in a particular pathology is often insufficient to provide a meaningful opportunity for therapeutic intervention. By comparison, comprehensive metabolic profiling would allow the physicians to see the whole panorama of metabolism and would allow an integrated, comprehensive characterization of the problem as well as an integrated, comprehensive approach to treatment.

Accordingly, there is a need for a method to comprehensively profile the metabolic abnormalities found in the fetus with Down Syndrome or other chromosome abnormalities. Ideally, a global screen should be performed for a wide range of metabolites at one time. The results of the global screen could then be utilized to construct a biochemical profile that suggests treatment pathways for possible fetal chromosomal abnormalities including Down Syndrome.

SUMMARY OF THE INVENTION

The present invention uses a sample of amniotic fluid to generate a comprehensive metabolic profile to diagnose chromosomal abnormalities in the fetus. The profile can be generated by several analytical techniques, and the components of the profile can be varied based on the clinical indication of interest. For example, a procedure similar to one described by Shoemaker and Elliott can be used to screen a specimen of amniotic fluid for metabolites.

Shoemaker and Elliott, *Automated screening of urine samples for carbohydrates, organic and amino acids after treatment with urease*, Journal of Chromatography, 562 (1991) 125-138, specifically incorporated herein by reference. A specimen of amniotic fluid is first obtained. For example, amniotic fluid may be taken from around the fetus during pregnancy. The specimen then is analyzed in a gas chromatograph/mass spectrophotometer (GC/MS).

The results of the GC/MS analysis are then used to generate the profile of the metabolites previously identified. The sample profile is compared with a control profile of metabolites that is representative of the normal levels of those metabolites. By analyzing the sample profile with respect to the normal profile, each metabolite that has a different level when compared with the normal level of that metabolite can be identified. Using the identified metabolites that have different values than the norm, a biochemical treatment may be prescribed that addresses the concentration, i.e., to increase or decrease, of each of those metabolites or the metabolism or physiology thereof. Using this method, an improved treatment for chromosomal abnormalities such as Down Syndrome may be prescribed by taking into account metabolic deficiencies on a global level rather than individually or in small groups.

DETAILED DESCRIPTION OF THE INVENTION

As previously discussed, a procedure similar to the procedure described by Shoemaker and Elliott may be used to screen an amniotic fluid specimen for metabolites. An amniotic fluid specimen is obtained from the fetus to be evaluated. For example, amniotic fluid may be obtained by placing a needle through the abdomen and uterine wall into the uterus and withdrawing the fluid with a syringe. It is preferable that the syringe not be lubricated with glycerol. The fluid may be separated from any unwanted cells by centrifugation.

If storage of the specimen is required, the specimen is preferably maintained at -20°C. Subsequently, the specimen is transferred to a metabolic screening laboratory where a GC/MS apparatus is located. If the laboratory is remotely located, the specimen is shipped on dry ice. The specimen is then thawed in the laboratory in preparation for the test.

The specimen is injected into the GC/MS apparatus in order to identify and measure the metabolites. All chemical constituents of the specimen are separated by their GC retention times. The identity and quantity of each chemical constituent is then determined by the MS. The MS preferably sweeps through all masses from 15-650 Daltons every two seconds.

A comprehensive metabolic profile is then generated that indicates the identity and quantity of each metabolite. From these levels, the activity of the relevant enzymes related to each metabolite can be inferred. The metabolites included in the report may include, but are not limited to, organic acids, amino acids, glycine conjugates, fatty acids, vitamins, neurotransmitters, drugs, drug metabolites, hormones, and carbohydrates.

The levels of the metabolites for the sample are then compared with the representative, normal level for each. The levels may be compared by examining mean levels and standard deviations for each metabolite. Alternatively, the levels may be compared using medians and a nonparametric analysis. By analyzing the relative levels of the metabolites as compared with the normal levels, a suitable biochemical treatment may be prescribed for a particular condition.

Most of the metabolites are indicator substrates. An indicator substrate is a substrate that is metabolized by an enzyme. When the enzyme is deficient or its activity is low, the indicator substrate accumulates. When the enzyme activity is high, the metabolite may be low. If the activity of an enzyme is low, vitamin or mineral cofactors can be supplemented. If the activity

of an enzyme is too high, it can be blocked or cofactors withheld. Some metabolites are vitamins or vitamin derivatives. If they are low, they can similarly be supplemented.

Any metabolite that may be included in a GC/MS report or analogous analytical technique may be analyzed by the method of the present invention. This method is also used to analyze a global metabolic profile to provide the biochemical equivalent of a complete physical exam. The method detects abnormalities through the comprehensive metabolic profiling which identifies multiple metabolic abnormalities. Each one of these abnormalities, when present by itself in a severe form, would cause mental retardation. Abnormalities such as Down Syndrome may then be modeled as the sum of a group of simpler abnormalities that may have an identifiable metabolic profile. Many of these simpler abnormalities may have treatments that are suggested by the results of the comprehensive metabolic profiling. Using the results of the comprehensive metabolic profile, chromosomal abnormalities may be identified and proper treatment may be suggested at an opportune time.

A key advantage of the method is the characterization of a disease state, a physiologic influence, or the effects of a drug. If a group of normal patients and a group of disease patients are characterized, the differences between the groups will form a biochemical characterization of the disease.

Example Profile Analysis

To diagnose a fetus for chromosomal abnormalities using the method of the present invention, a metabolic profile must first be generated that is representative of the metabolite levels in an average patient suffering from the chromosomal abnormality that is to be diagnosed. Using the aforementioned GC/MS procedure, a metabolic profile for a group of 23 Down

Syndrome patients was generated. The following tables represent the Down Syndrome metabolic profile separated into different metabolite groups. The tables also include results for a group of 41 normal patients, generated by the GC/MS procedure, for comparison purposes. Since the comparison data for a chromosomal abnormality may be analyzed by examining either the mean levels of the metabolites or the median levels of the metabolites, two tables are presented for each metabolite grouping with one table presenting mean levels and the other presenting median levels (i.e., a nonparametric analysis). Within each table, the Mann-Whitney p-value and the normal value v. abnormal value t-Test value are presented for each metabolite. Additionally, the standard deviation (S.D.) is presented for each metabolite in the tables that present the mean levels of the metabolites rather than the median levels.

Table 1 illustrates a typical metabolic profile for the mean level of fatty acids within a population of Down Syndrome patients and a population of normal patients:

Fatty Acid Compound	t-Test	Normal Mean	S.D.	Down Syndrome Mean	S.D.
LAURIC ACID	0.1874	0.075	0.2897	0.3260	0.8608
MYRISTIC ACID	0.5022	2.2875	3.0146	3.0434	4.8286
PALMITOLEIC ACID	0.8122	0.0712	0.2428	0.0869	0.2559
PALMITIC ACID	0.6203	23.0625	30.183	18.7173	34.919
LINOLEIC ACID	0.6124	5.45	7.1313	7.7173	20.4982
LINOLENIC ACID	0.4651	3.3775	5.8258	5.4956	12.9802
OLEIC ACID	0.6296	39.8	65.011	30.4130	78.4342
STEARIC ACID	0.6709	3.1125	3.6837	2.6739	4.0469
ARACHIDONIC ACID	0.7258	0.5675	2.3453	0.4086	1.2291
EICOSAPENTAENOIC	0.7013	0.1237	0.3415	0.1	0.1422

Table 1

Table 2 illustrates a typical metabolic profile for the median level of fatty acids within a population of Down Syndrome patients and a population of normal patients:

Fatty Acid Compound	p-value	Normal Median	Down Syndrome Median
LAURIC ACID	0.049	0	0

MYRISTIC ACID	0.575	1.25	1.5
PALMITOLEIC ACID	0.576	0	0
PALMITIC ACID	0.342	11.75	10.5
LINOLEIC ACID	1	2.25	2.5
LINOLENIC ACID	0.91	0.675	0.8
OLEIC ACID	0.391	11.5	10
STEARIC ACID	0.564	1.5	1.5
ARACHIDONIC ACID	0.103	0	0
EICOSAPENTAENOIC	0.361	0	0

Table 2

Table 3 illustrates a typical metabolic profile for the mean level of simple sugars within a population of Down Syndrome patients and a population of normal patients:

Simple Sugars		Normal		Down Syndrome	
Compound	t-Test	Mean	S.D.	Mean	S.D.
THREITOL	0.7620	7.8875	4.6979	7.5217	4.5263
ERYTHRITOL	0.1094	6.9875	5.3594	4.5869	5.7537
ARABINOSE	0.5616	0.6375	0.6503	0.7391	0.6719
FUCOSE	0.2285	0.3975	1.1847	0.1630	0.2001
RIBOSE	0.3449	0.6425	0.3888	0.7652	0.5394
ERYTHRITOL.2	0.2743	9.3625	5.3011	11.1956	6.8286
FRUCTOSE	0.5252	27.1	43.9131	17.7391	61.5203
GLUCOSE mg/dL	0.7164	21.3	24.4613	19.1739	20.8744
GALACTOSE	0.5750	1856.16	2477.33	1545.69	1856.03
MANNOSE	0.2175	3.7625	3.2874	2.8913	2.2409
N-AC-GLUCOSAMINE	0.5290	0.3225	0.4437	0.2652	0.2740
LACTOSE	0.4813	0.625	0.9919	0.8043	0.9503
MALTOSE	0.3696	0.6125	1.0830	0.8913	1.2243
XYLITOL	0.7618	6.465	8.2362	5.9804	4.3894
ARABINITOL	0.1907	0.4862	1.5326	0.1586	0.2097
RIBITOL	0.9760	0.195	0.4903	0.1978	0.2524
ALLOSE	0.3697	0.7512	1.7308	0.4869	0.4873
GLUCURONIC ACID	0.3784	59.7912	137.818	36.8978	66.2477
GALACTONIC ACID	0.9373	149.55	776.895	162.347	507.218
GLUCONIC ACID	0.8097	0.3587	0.7182	0.3913	0.3466
GLUCARIC	0.3784	0.06	0.0968	0.0913	0.1512
MANNITOL	0.8416	45.9612	120.018	50.9195	75.9496
DULCITOL	0.4140	0.1312	0.6863	0.0413	0.0468
SORBITOL	0.3269	0.15	0.1870	0.2086	0.2452
INOSITOL	0.9174	26.8425	26.0067	27.4782	21.6581
SUCROSE	n/a	0	0	0	0
METANEPHRINE mg/DL	0.3281	0	0	0.0021	0.0104

Table 3

Table 4 illustrates a typical metabolic profile for the median level of simple sugars within a population of Down Syndrome patients and a population of normal patients:

Simple Sugars Compound	p-value	Normal Median	Down Syndrome Median
THREITOL	0.627	7.25	6.5
ERYTHRITOL	0.057	7	1.5
ARABINOSE	0.504	0.5	0.5
FUCOSE	0.192	0.05	0.1
RIBOSE	0.663	0.675	0.65
ERYTHRITOL.2	0.431	8	8.5
FRUCTOSE	0.102	1.5	1.5
GLUCOSE mg/dL	0.716	12.5	11.5
GALACTOSE	0.498	1056.5	692
MANNOSE	0.352	3	3
N-AC-GLUCOSAMINE	0.317	0.1	0.2
LACTOSE	0.204	0.5	0.5
MALTOSE	0.231	0	0.5
XYLITOL	0.602	4.3	5.05
ARABINITOL	0.455	0.1	0.1
RIBITOL	0.099	0	0.1
ALLOSE	0.769	0.3	0.35
GLUCURONIC ACID	0.797	4.65	1.8
GALACTONIC ACID	0.853	8	9
GLUCONIC ACID	0.085	0.175	0.35
GLUCARIC	0.471	0	0.05
MANNITOL	0.264	0.325	0.7
DULCITOL	0.053	0	0.05
SORBITOL	0.336	0.1	0.15
INOSITOL	0.462	16.75	27.5
SUCROSE	1	0	0
METANEPHRINE mg/dL	0.187	0	0

Table 4

Table 5 illustrates a typical metabolic profile for the mean level of amino acids and glycine conjugates within a population of Down Syndrome patients and a population of normal patients:

Amino Acids and Glycine Conjugates Compound	t-Test	Normal Mean	Normal S.D.	Down Syndrome Mean	Down Syndrome S.D.
PROPIONYL GLY	0.9948	0.2225	0.6289	0.2217	0.2848
BUTYRYL GLYCINE	0.8212	0.2213	0.6913	0.2565	0.5292
HEXANOYL GLYCINE	0.4152	0.3938	1.3668	0.2087	0.3006

PHENYL PROP GLY	0.7414	0.5000	2.3993	0.3543	1.0615
SUBERYL GLYCINE	0.5090	0.0300	0.0890	0.0457	0.0903
ISOVALERYL GLY	0.9786	0.4650	1.9601	0.4543	1.1773
TIGLY GLY	0.4076	0.3625	0.7605	0.2457	0.3447
BETA MET CROT GLY	0.4827	1.2750	4.3378	0.7696	0.9522
GLYCINE	0.3641	276.2250	199.0262	229.2826	193.7927
ALANINE	0.0337	455.3000	491.5694	202.5870	412.2851
SARCOSINE	0.2933	1.0988	1.2466	0.7826	1.0703
BETA-ALANINE	0.9038	17.2700	11.8828	16.9109	10.9422
B-AMINOISOBUTYRIC	0.2457	2.2538	2.7931	1.6609	1.1794
SERINE	0.2684	19.7875	17.7825	15.1087	14.8561
PROLINE	0.0385	318.4875	322.0118	186.5435	172.6874
HYDROXY PROLINE	0.1647	81.1000	50.1975	105.9565	74.7918
HYDROXY LYSINE	0.9268	0.7413	1.2271	0.7152	0.9819
ASPARTIC ACID	0.1032	6.7913	5.0833	11.1761	11.8518
ASPARAGINE	0.7315	0.6338	0.7522	0.6913	0.5618
N-AC ASPARTIC	0.8949	0.1800	0.6900	0.1978	0.3761
ORNITHINE	0.3084	40.8000	29.5179	32.6522	30.6162
GLUTAMIC ACID	0.9576	261.4500	204.4625	258.6522	197.4870
GLUTAMINE	0.1047	1.0250	3.0633	3.5870	6.9407
PIPECOLIC ACID	0.7858	3.4488	2.7644	3.7065	3.9971
LEUCINE	0.0073	231.5000	181.3793	126.5652	117.7761
KETO LEUCINE	0.8192	53.0900	39.8797	50.0196	56.3368
VALINE	0.0440	383.8125	302.1702	239.1087	246.4417
KETO-VALINE	0.9095	13.8475	12.2042	14.2370	13.4663
ISOLEUCINE	0.0651	103.6875	86.9055	67.2609	65.4288
KETO-ISOLEUCINE	0.7580	7.5625	7.5049	8.2000	8.0510
LYSINE	0.7142	366.5500	287.9107	341.2174	247.3257
HISTIDINE	0.8280	14.7000	12.2138	14.0217	11.6643
THREONINE	0.2161	292.3125	277.0502	217.4348	195.7553
HOMOSERINE	0.5177	0.3688	0.3549	0.4261	0.3250
METHIONINE	0.5314	34.8375	30.2578	40.2609	34.2282
CYSTEINE	0.1124	152.9625	103.4822	212.1957	155.5338
HOMOCYSTEINE	0.0690	0.0625	0.1436	0.1565	0.2139
CYSTATHIONINE	0.2705	0.1288	0.2287	0.2087	0.2957
HOMOCYSTINE	0.7456	0.0688	0.1846	0.0565	0.1131
CYSTINE	0.6096	0.3138	0.7375	0.2457	0.3030
PHENYLALANINE	0.2054	144.6500	79.8540	180.2174	117.4317
TYROSINE	0.7914	107.1750	98.7430	100.6087	91.7593
TRYPTOPHAN	0.9187	10.3875	10.1908	10.1087	10.4870

Table 5

Table 6 illustrates a typical metabolic profile for the median level of amino acids and glycine conjugates within a population of Down Syndrome patients and a population of normal patients:

Amino Acids and Glycine Conjugates Compound	p-value	Normal Median	Down Syndrome Median
PROPIONYL GLY	0.108	0	0.15
BUTYRYL GLYCINE	0.02	0	0.1
HEXANOYL GLYCINE	0.011	0	0.1
PHENYL PROP GLY	0.933	0	0
SUBERYL GLYCINE	0.148	0	0
ISOVALERYL GLY	0.005	0	0.1
TIGLY GLY	0.976	0.125	0.1
BETA MET CROT GLY	0.41	0.175	0.55
GLYCINE	0.357	280	249.5
ALANINE	0.069	312.25	2
SARCOSINE	0.399	0.7	0.25
BETA-ALANINE	1	16.025	18.25
B-AMINOISOBUTYRIC	0.937	1.35	1.4
SERINE	0.247	17.25	13
PROLINE	0.265	199.5	129
HYDROXY PROLINE	0.214	68.5	86.5
HYDROXY LYSINE	0.552	0.225	0.3
ASPARTIC ACID	0.454	6.35	8
ASPARAGINE	0.399	0.475	0.45
N-AC ASPARTIC	0.006	0	0.05
ORNITHINE	0.138	34.25	24
GLUTAMIC ACID	0.926	212.75	249
GLUTAMINE	0.002	0.5	1
PIPECOLIC ACID	0.903	2.8	2.75
LEUCINE	0.036	221.25	93.5
KETO LEUCINE	0.457	49.725	36.3
VALINE	0.085	368.5	176.5
KETO-VALINE	0.972	11	9.9
ISOLEUCINE	0.123	93.5	51
KETO-ISOLEUCINE	0.689	6.125	6.25
LYSINE	0.627	280.25	270
HISTIDINE	0.732	11.5	11
THREONINE	0.265	236.75	155
HOMOSERINE	0.405	0.3	0.4
METHIONINE	0.638	33.35	38.7
CYSTEINE	0.104	144	200
HOMOCYSTEINE	0.01	0	0.1
CYSTATHIONINE	0.09	0.05	0.1
HOMOCYSTINE	0.114	0	0
CYSTINE	0.115	0.075	0.15
PHENYLALANINE	0.467	138	150.5
TYROSINE	0.663	66	67
TRYPTOPHAN	0.869	6.25	4.5

Table 6

Table 7 illustrates a typical metabolic profile for the mean level of neurotransmitters within a population of Down Syndrome patients and a population of normal patients:

Neurotransmitters		Normal		Down Syndrome	
Compound	t-Test	Mean	S.D.	Mean	S.D.
GABA	0.9990	0.8063	0.7186	0.8065	0.8286
HOMOVANILLIC ACID	0.5579	0.1950	0.3004	0.2609	0.4824
NORMETANEPHRINE	0.0388	0.0013	0.0079	0.0391	0.0825
VANILLYLMANDELIC	0.0849	0.0038	0.0175	0.0152	0.0279
METANEPHRINE	0.5954	0.6488	2.2671	0.4022	1.3971
5-HIAA	0.5821	0.0100	0.0258	0.0065	0.0229
MHPG	0.5438	0.0038	0.0175	0.0065	0.0172
ETHANOLAMINE	0.6039	63.3375	46.5176	56.7826	48.7374

Table 7

Table 8 illustrates a typical metabolic profile for the median level of neurotransmitters within a population of Down Syndrome patients and a population of normal patients:

Neurotransmitters		Normal	Down Syndrome
Compound	p-value	Median	Median
GABA	0.539	0.75	0.45
HOMOVANILLIC ACID	0.663	0.1	0.15
NORMETANEPHRINE	0.001	0	0
VANILLYLMANDELIC	0.019	0	0
METANEPHRINE	0.707	0.075	0.1
5-HIAA	0.489	0	0
MHPG	0.279	0	0
ETHANOLAMINE	0.753	65.5	65

Table 8

Table 9 illustrates a typical metabolic profile for the mean level of nutritionals within a population of Down Syndrome patients and a population of normal patients:

Nutritionals		Normal		Down Syndrome	
Compound	t-Test	Mean	S.D.	Mean	S.D.
FORMIMINOGLUTAMIC ACID	0.0033	1.4877	0.0462	0.8829	0.7441
4-PYRIDOXIC ACID	0.4924	1.2863	1.8158	1.0370	1.0489
PANTOTHENIC ACID	0.8733	299.3500	527.7872	278.8043	466.6548
XANTHURENIC ACID 407	0.3163	0.0400	0.2214	0.0043	0.0144
KYNURENINE	0.8218	1.2075	1.6656	1.1283	1.1077
QUINOLINIC	0.8759	0.1913	1.0503	0.2261	0.7077
OROTIC ACID	0.1702	0.0133	0.0502	0.0543	0.1344

D-AM LEVULINIC	0.1741	11.3150	23.8991	5.8217	6.0014
3-METHYL HISTIDINE	0.8620	1.6000	1.9553	1.5217	1.5556
NIACINAMIDE	0.8650	0.8825	4.2032	0.7587	1.3854
PSEUDOURIDINE	0.7538	0.2875	0.4220	0.3261	0.4910
2-DEOXYTETRONIC	0.2681	1.2250	1.1033	1.5435	1.0757
P-HO-PHEN-ACETIC	0.5254	0.1125	0.2399	0.1522	0.2352
XANTHINE	0.6903	0.0488	0.1508	0.0630	0.1272
UROCANIC ACID	0.9655	0.0625	0.3240	0.0652	0.1722
ASCORBIC ACID	0.3415	0.1000	0.4961	0.0217	0.1043
GLYCEROL	0.4712	36.6663	28.3624	31.4870	26.5956

Table 9

Table 10 illustrates a typical metabolic profile for the median level of nutritionals within a population of Down Syndrome patients and a population of normal patients:

Nutritionals Compound	p-value	Normal Median	Down Syndrome Median
FORMIMINOGLUTAMIC ACID	0.007	1.3425	0.9119
4-PYRIDOXIC ACID	0.839	0.8	0.8
PANTOTHENIC ACID	0.932	149.5	151.5
XANTHURENIC ACID 407	0.822	0	0
KYNURENINE	0.303	0.425	0.8
QUINOLINIC	0.199	0	0
OROTIC ACID	0.189	0	0
D-AM LEVULINIC	0.587	2.85	2.6
3-METHYL HISTIDINE	0.712	1	1
NIACINAMIDE	0.044	0	0.25
PSEUDOURIDINE	0.775	0	0
2-DEOXYTETRONIC	0.214	1	1.5
P-HO-PHEN-ACETIC	0.39	0	0
XANTHINE	0.653	0	0
UROCANIC ACID	0.279	0	0
ASCORBIC ACID	0.877	0	0
GLYCEROL	0.539	35.525	31.85

Table 10

Table 11 illustrates a typical metabolic profile for the mean level of organic acids within a population of Down Syndrome patients and a population of normal patients:

Organic Acids Compound	t-Test	Normal Mean	S.D.	Down Syndrome Mean	S.D.
LACTIC ACID uM/L	0.3580	7455.3375	6476.7984	6096.8478	5027.6119
PYRUVIC ACID	0.0563	16.1375	22.5374	7.9783	10.5760
GLYCOLIC ACID	0.3684	25.2375	65.5558	43.5435	82.6529

ALPHA-OH-BUTYRIC	0.0965	21.4750	22.4214	11.6304	22.0337
OXALIC ACID	0.0239	12.7000	21.4652	34.8913	41.5667
4-OH-BUTYRIC	0.4037	0.1000	0.4114	0.1957	0.4457
HEXANOIC ACID	0.9894	11.1375	21.0619	11.0652	20.5234
5-HYDROXYCAPROIC	0.5482	0.2100	0.9129	0.3239	0.5825
OCTANOIC	0.9308	0.6625	1.8271	0.6304	1.0894
BETA-LACTATE	0.0313	11.9625	13.0852	6.1522	7.8327
SUCCINIC ACID	0.7615	1.2125	1.5603	1.3261	1.3366
GLUTARIC ACID	0.3675	0.5588	2.2167	0.2261	0.4970
2-OXO-GLUTARATE	0.1486	6.1250	13.4273	11.9130	15.8592
FUMARIC	0.8900	0.1275	0.3629	0.1174	0.2146
MALEIC	0.0986	2.7875	3.1928	4.7348	4.9316
MALIC ACID	0.4096	2.0000	2.7087	2.5587	2.4817
ADIPIC ACID	0.8252	0.1375	0.5126	0.1174	0.1940
SUBERIC ACID	0.7434	0.1925	0.3964	0.2196	0.2557
SEBACIC ACID	0.0816	0.6875	0.8276	0.3413	0.6933
GLYCERIC ACID	0.6070	28.0000	22.2955	25.2609	18.9406
BETA-OH-BUTYRIC	0.4624	61.4750	53.3212	50.2174	60.5152
METHYLSUCCINIC	0.1623	0.1775	0.7137	0.5087	0.9728
METHYLMALONIC	0.9946	25.3988	23.5841	25.4348	18.3025
ETHYLMALONIC	0.3967	28.3250	65.1132	16.7174	42.6016
HOMOGENTISIC ACID	0.3281	0.0138	0.0519	0.0370	0.1047
PHENYLPYRUVIC ACID	0.0254	0.0375	0.0749	0.1435	0.2063
SUCCINYLACETONE	0.9787	0.2600	0.5466	0.2630	0.3549
3-OH-ISOVALERIC	0.5760	3.5813	3.9367	3.0652	3.2301
PHOSPHATE mg/dL	0.6839	1.0363	1.2176	0.9174	1.0422
CITRIC ACID	0.9542	36.7000	47.5228	37.3261	37.4884
HIPPURIC ACID	0.0613	21.4125	24.8725	12.9783	9.6218
URIC ACID mg/dL	0.5409	0.4200	0.7250	0.3391	0.3086

Table 11

Table 12 illustrates a typical metabolic profile for the median level of organic acids within a population of Down Syndrome patients and a population of normal patients:

Organic Acids Compound	p-value	Normal Median	Down Syndrome Median
LACTIC ACID uM/L	0.521	5563.5	5138
PYRUVIC ACID	0.311	5.25	5
GLYCOLIC ACID	0.668	12.5	13
ALPHA-OH-BUTYRIC	0.046	17.25	1
OXALIC ACID	0.007	5	23.5
4-OH-BUTYRIC	0.103	0	0
HEXANOIC ACID	0.731	6.25	4
5-HYDROXYCAPROIC	0.009	0	0.05
OCTANOIC	0.643	0	0
BETA-LACTATE	0.198	6	4.5

SUCCINIC ACID	0.433	0.5	1
GLUTARIC ACID	0.095	0	0.05
2-OXO-GLUTARATE	0.034	1	5
FUMARIC	0.175	0	0.05
MALEIC	0.108	1.525	2.5
MALIC ACID	0.252	0.975	1.6
ADIPIC ACID	0.009	0	0.05
SUBERIC ACID	0.162	0.05	0.1
SEBACIC ACID	0.13	0.35	0.1
GLYCERIC ACID	0.814	25.25	26
BETA-OH-BUTYRIC	0.414	66.25	33
METHYLSUCCINIC	0.002	0	0.15
METHYLMALONIC	0.563	21.3	24.3
ETHYLMALONIC	0.407	6.125	2.8
HOMOGENTISIC ACID	0.218	0	0
PHENYLPYRUVIC ACID	0.017	0	0.05
SUCCINYLACETONE	0.188	0.05	0.1
3-OH-ISOVALERIC	0.881	2.375	1.45
PHOSPHATE mg/dL	0.949	0.65	0.7
CITRIC ACID	0.38	13	22.5
HIPPURIC ACID	0.287	11.25	9
URIC ACID mg/dL	0.426	0.2	0.25

Table 12

If a metabolic profile of a specific patient reflected the same profile as the Down Syndrome group, the method of the present invention could be used to diagnose and suggest treatment for this patient. In the example profile data, formiminoglutamate (FIGLU) in Down Syndrome (Mean = 0.8829, Median = 0.9119) is decreased from normal (Mean = 1.4877, Median = 1.3425). (See Tables 9 and 10). FIGLU is a metabolite of histidine, which contributes a mono-carbon to tetrahydrofolate. In folate deficiency, FIGLU accumulates because there is little folate to accept the mono-carbon. Therefore, folate deficiency results in a shortage of mono-carbon tetrahydrofolate. Additionally, mono-carbons are necessary to synthesize biologically important molecules. Mono-carbon tetrahydrofolate is a major source of mono-carbons used in cellular biosynthesis. Mono-carbons are required to synthesize purines (e.g. adenine & guanine), components of DNA, RNA, and other important molecules. In Down Syndrome, purine synthesis is accelerated. There are signs of mono-carbon shortage but folate is

not deficient. In our data, we see that FIGLU is reduced. This probably reflects an increased demand for mono-carbons due to accelerated purine synthesis. Therefore, a treatment of mono-carbon supplements could be prescribed for this patient.

Another metabolite that should be addressed according to this data is normetanephrine. (See Tables 7 and 8). Normetanephrine is a metabolite of the neurotransmitter norepinephrine (NE). NE requires a mono-carbon to be metabolized to epinephrine. Mono-carbon shortage explains the finding of elevated normetanephrine in Down Syndrome (Mean = 0.039, Median = 0) compared to normal amniotic fluid (Mean =.0013, Median = 0).

Cystathionine beta synthase (CBS) is an enzyme whose gene is on chromosome 21. This enzyme catalyzes the reaction of serine and homocysteine to cystathionine. Subsequently cystathionine is converted to cysteine. Since this enzyme will be present in excess in a Down Syndrome patient, we would expect a reduction in the substrates serine and homocysteine and an increase in the products cystathionine and cysteine. Referring to Tables 5 and 6, serine in a Down Syndrome patient (Mean = 15.1087, Median = 13) is reduced from levels in a normal patient (Mean = 19.7875, Median = 17.25). Cystathionine is greater in a Down Syndrome patient (Mean = 0.2087, Median = 0.1) than in a normal patient (Mean = 0.1288, Median = 0.05). Cysteine is greater in a Down Syndrome patient (Mean = 212.1957, Median = 200) than in a normal patient (Mean = 152.9625, Median = 144). Homocysteine is greater in a Down Syndrome patient (Mean = 0.1565, Median = 0.1) than in a normal patient (Mean = 0.0625, Median = 0.0).

Prior to the method of the current invention, the aforementioned results would ordinarily be difficult to comprehend. However, pursuant to the invention, the examination of the results from the nutritional molecule FIGLU and from the neurotransmitter normetanephrine enables a

treatment to be suggested for these conditions. These results indicate a shortage of mono-carbons. Homocysteine would normally be methylated to methionine and then converted to s–adenosyl-methionine (SAM). SAM is a major source of mono-carbons in the cell. Since such mono-carbons are in short supply, homocysteine accumulates due to the lack of mono-carbons necessary to methylate homocysteine to methionine. Again, this suggests that a supplement of mono-carbons should be prescribed. Thus, a treatment regimen that supplements mono-carbons is prescribed to a patient pursuant to the data provided by the broad metabolic profile analysis described herein.

Holocarboxylase synthase is located on chromosome 21. This enzyme causes metabolic activation of the vitamin biotin. The data here suggest that some biotin dependent enzymes are accelerated in Down Syndrome. First, beta-lactate, also known as 3-hydroxy-propionate, is a marker for propionyl COA carboxylase, a biotin dependent enzyme. Beta-lactate in a Down Syndrome patient (Mean = 6.1522, Median = 4.5) is lower than in a normal patient (Mean = 11.9625, Median = 6). (See Tables 11 and 12). Additionally, leucine is lower in a Down Syndrome patient (Mean = 126.5652, Median = 93.5) than in a normal patient (Mean = 231.5, Median = 221.25). (See Tables 5 and 6). Isoleucine is also lower in a Down Syndrome patient (Mean = 67.2609, Median = 51) than in a normal patient (Mean = 103.6875, Median = 93.5). Catabolism of both isoleucine and leucine require biotin.

These results suggest that patients with Down Syndrome have an acceleration of biotin-dependent pathways due to an increase in gene dosage from holocarboxylase synthase. Biotin is generally thought to be non-toxic. If these accelerated pathways were harmful, a patient may benefit from a biotin restriction pursuant to the aforementioned data.

Another treatment that is suggested by the method of globally analyzing a complete metabolic profile of this patient for chromosomal abnormalities is a tetra-hydra-biopterin supplement. Phenylpyruvate (phenylpyruvic acid) is elevated in a Down Syndrome patient (Mean = 0.1435, Median = 0.05) over a normal level (Mean = 0.0375, Median = 0). (See Tables 11 and 12). Phenylalanine is increased in a Down Syndrome patient (Mean = 180.2174, Median = 150.5) over a normal level (Mean = 144.65, Median = 138). (See Tables 5 and 6). These two metabolites are elevated in phenylketonuria (PKU), a disease which causes mental retardation. The enzyme affected is phenylalanine hydroxylase. The enzyme requires tetrahydrabiopterin, thought to be deficient in Down Syndrome patients. The aforementioned data suggests that tetrahydrabiopterin deficiency is present in Fetal Down Syndrome and should be treated by prescribing tetrahydrabiopterin supplements.

Furthermore, analysis of the metabolite data may suggest a vitamin B6 supplement. Turning again to Tables 11 and 12, oxalic acid (oxalate) in a Down Syndrome patient (Mean = 34.8913, Median = 23.5) is elevated over normal levels (Mean = 12.7, Median = 5). This characteristic suggests a functional deficiency of vitamin B6, also known as pyridoxine. Therefore, a vitamin B6 supplement should be prescribed.

The above findings can be assembled to give an itemized biochemical characterization of fetal Down Syndrome. Some of the biochemical abnormalities are treatable. As shown below, several types of treatment may be prescribed based on the biochemical characterization of Down Syndrome discussed above.

ABNORMALITY	PRESCRIBED SUPPLEMENT(S)
Mono-carbon Shortage	Folate, B12, Mono-carbon donors (ex. Methionine, Betaine, Choline, Dimethylglycine)
Increased Homocysteine	Folate, B12, Mono-carbon donors (ex. Methionine, Betaine, Choline, Dimethylglycine)
Increased NE	Folate, B12, Mono-carbon donors (ex. Methionine, Betaine, Choline, Dimethylglycine)
Functional B6 deficiency	B6
Reduced serine	Serine
Tetra-hydro-biopterin deficient	Tetra-hydro-biopterin

With the current method, a simultaneous assessment of all metabolites improves the assessment of individual metabolites and suggests treatment that might have otherwise been overlooked.

The particular examples set forth herein are instructional and should not be interpreted as limitations on the applications to which those of ordinary skill are able to apply this invention. Modifications and other uses are available to those skilled in the art which are encompassed within the spirit of the invention as defined by the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method of characterizing a chromosomal abnormality in a fetus by performing a comprehensive biochemical analysis of a specimen of amniotic fluid comprising:

obtaining a comprehensive profile of metabolites in the specimen of amniotic fluid,

comparing the profile with a control profile of metabolites that is representative of normal levels of metabolites,

analyzing the profile with respect to the normal profile by identifying each metabolite that has a different level when compared with the normal level of that metabolite,

generating a biochemical characterization of the abnormality, and

prescribing a biochemical treatment for each metabolite that has a different level when compared with the normal level of that metabolite.

2. The method of Claim 1 wherein comparing the profile with respect to the normal profile is accomplished by comparing mean levels and standard deviations for each metabolite.

3. The method of Claim 1 wherein comparing the profile with respect to the normal profile is accomplished by comparing median levels using a nonparametric analysis for each metabolite.

4. The method of Claim 1 wherein Down Syndrome is the chromosomal abnormality that is diagnosed.

5. The method of Claim 1 wherein the metabolite is chosen from the group consisting of organic acids, amino acids, neurotransmitters, fatty acids, glycine conjugates, drugs, drug metabolites, hormones, vitamins, and carbohydrates.

6. The method of Claim 1 wherein the metabolites comprise multiple categories of metabolite groups that are analyzed simultaneously.

7. A method of performing a comprehensive biochemical analysis of a specimen of amniotic fluid in order to characterize a chromosomal abnormality in a fetus comprising:

obtaining a comprehensive profile of metabolites in the specimen of amniotic fluid,

comparing the profile with a control profile of metabolites that is representative of normal levels of the reported metabolites,

analyzing the profile with respect to the normal profile by identifying each metabolite that has a different level when compared with the normal level of that metabolite,

inferring an activity level for an enzyme that corresponds to the identified metabolite,

inferring a cofactor level based on the activity level for the enzyme,

generating a global biochemical characterization of the abnormality, and

prescribing a biochemical treatment for each metabolite that has a different level when compared with the normal levels.

8. A method of characterizing a chromosomal abnormality in a fetus by performing a comprehensive biochemical analysis of a specimen of amniotic fluid comprising:

obtaining a comprehensive profile of metabolites in the specimen of amniotic fluid,

comparing the profile with a control profile of metabolites that is representative of levels of metabolites in patients suffering from the chromosomal abnormality,

analyzing the profile with respect to the chromosomal abnormality profile by identifying each metabolite that has a same level when compared with the abnormal level of that metabolite, and

prescribing a biochemical treatment for each metabolite that has a same level when compared with the abnormal level of that metabolite.

9. The method of Claim 8 wherein comparing the profile with respect to the abnormal profile is accomplished by comparing mean levels and standard deviations for each metabolite.

10. The method of Claim 8 wherein comparing the profile with respect to the abnormal profile is accomplished by comparing median levels using a nonparametric analysis for each metabolite.

11. The method of Claim 8 further comprising:

inferring an activity level for an enzyme that corresponds to an identified metabolite having a same level as a metabolite in the abnormal profile, and

inferring a cofactor level based on the activity level for the enzyme.

ABSTRACT

A specimen of an amniotic fluid is obtained and analyzed by GC/MS in order to generate a comprehensive metabolic profile. The profile is analyzed by comparing the levels of metabolites with normal levels of those compounds. Specific treatment is then prescribed for the metabolite levels that differ from the norm. These metabolites that are present in different levels than a normal specimen may be indicative of chromosomal abnormalities such as Down Syndrome. The method of the present invention is used to model the complex problem of a chromosomal abnormality as the sum of several simpler problems that may be treatable. The comprehensive metabolic profile is used to detect chromosomal abnormalities, to suggest treatments for fetal chromosomal abnormalities, and to monitor their effectiveness.